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Abstract

Synthetic glucocorticoids are frequently used antenatally in order to reduce morbidity and mortality in babies born preterm and have been used in the management of fetuses known to be at risk of congenital adrenal hyperplasia. Although such treatment has short term advantages, evidence suggests that it can affect health in later life. Several studies have reported negative consequences of prenatal exposure to the synthetic glucocorticoid dexamethasone on offspring behaviour in humans and in animal models, in association with changes in brain structure, hypothalamic-pituitary-adrenal axis function, neurotransmitter pathways, gene transcription and epigenetic regulation. These studies also highlight the importance of timing and tissue/organ- and sex-specific effects of prenatal glucocorticoid exposure. Here we review the evidence from human and animal studies that links prenatal synthetic glucocorticoid exposure with an increased risk for neurodevelopmental disorders.

Introduction

The concept of 'early life programming' is used to describe the association between environmental factors that occur during early life (pre- or postnatal) and an increased risk of diseases in later life [1]. According to this theory, environmental insults occurring at critical developmental periods can permanently alter the physiology and function of developing organs, resulting in lifelong consequences for health [2]. Such developmental plasticity has been proposed to help the unborn animal to optimize its body systems for better survival in a predicted adverse postnatal environment, however if the expected environment does not match the real postnatal environment, it may lead to malfunction and disease [3].

Physiological responses to environmental adversities are mediated by the hypothalamic-pituitary-adrenal (HPA) axis, which controls circulating glucocorticoid (GC) concentrations. Dysregulation of the HPA axis is associated with several neurodevelopmental disorders including autism [4] and schizophrenia [5]. Circulating GC levels are lower in the fetus compared to its mother as a consequence of the action of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β- HSD2) at the fetoplacental barrier, which catalyses the conversion of active GC (e.g. cortisol in humans and corticosterone in rodents) into their inactive forms (cortisone and 11-dehydrocorticosterone), and thus protects the fetus. Nevertheless, this barrier is not complete, and high levels of maternal GC can overcome this enzymatic protection [6]; indeed, fetal exposure to elevated levels of maternal GC, as a consequence of maternal stress during pregnancy, may impact on the developing brain and result in permanent changes in brain function [7].

In addition to exposure to increased endogenous GC levels following maternal stress, fetuses can also be exposed to high levels of synthetic GC (sGC), e.g. dexamethasone (DEX) or betamethasone, which are frequently administrated to mothers at risk of preterm delivery because of the clear benefits of GC on organ maturation, particularly the fetal lung [8]. sGC are additionally used in the management of fetuses at risk of (or diagnosed with) congenital adrenal hyperplasia to suppress fetal androgen production [9]. Such sGC are poor substrates for the 11β-HSD2 enzyme, and can readily cross the placental barrier. In this short review, we focus on the effects of prenatal sGC exposure on the brain, including effects on brain function and the epigenome.

Effects of prenatal glucocorticoid exposure on behaviour

Although prenatal DEX exposure has been associated with a decrease in the rate of cerebral palsy in preterm infants [10], it has also been associated with an increased risk of anxiety, hyperactivity and distractibility in preterm and term born children [10–12], with females being more susceptible to stress than males [11]. Nevertheless, reports on the outcomes of GC overexposure during early life are sometimes contradictory; for example, a long-term follow-up study of Swedish children who were prenatally treated with DEX showed no changes in psychopathology, behavioral problems or adaptive functioning, on the contrary they were described by their parents as being more sociable than the controls [13].

The difficulties in undertaking long-term follow-up studies in humans have led to the development of animal models for the study of effects of prenatal exposure to sGC. The majority of these models use DEX administration to pregnant dams, however the programmed phenotypes depend critically on the timing of exposure. In marmoset monkeys, juveniles exposed to early DEX (gestation day 42-48; gestation length ~148 days) were less sociable and more motivated to obtain a palatable reward, whereas later DEX treatment (from Day 90-96 of gestation) enhanced reversal learning of stimulus association. Offspring of both treatment groups showed a deficit in skilled motor reaching, however the effect was stronger with late DEX treatment [14].

In rats, DEX administration during the last week of gestation increased acoustic startle responses (ASR) in animals which had undergone prior blood sampling [15], suggesting that DEX offspring are more susceptible to anxiety [15]. Other studies also show DEX-exposed rats spend less time on the open arms of an elevated plus maze (EPM) [16,17] and spend less time in the central area of an open field test (OFT) with increased defecation and decreased exploratory activity [16,18,19], consistent with a phenotype of hyperanxiety. However, experimental findings vary in this field. Very recently, we have shown that DEX-exposed male rats demonstrate altered cognition in the Morris water maze [20], but no behavioural differences on an EPM or OFT (Table 1).

Overall, these studies suggest that prenatal DEX exposure programs an anxious phenotype, however the timing and dose of the DEX administered to the pregnant dams and the age and sex of the offspring may be crucial in determining any later effects.

Prenatal sGC exposure and brain development

sGC can exert effects by binding to the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), although they have a higher affinity for GR. Glucocorticoid receptors (GR) are widely expressed in the brain from early in prenatal development [21,22], especially in the hippocampus [21], explaining why prenatal exposure to sGC could have a significant impact on brain development [23]. Indeed, prenatal or postnatal sGC administration in rodents and humans significantly reduces brain weight [24,25] and produces structural changes in brain regions including the prefrontal cortex (PFC) [26], amygdala [27], hippocampus [28] and striatum [29] (Figure 1).

The hippocampus may be particularly vulnerable to prenatal sGC exposure: several studies report that prenatal DEX exposure increases apoptosis in hippocampal structures (Dentate Gyrus and Cornu Ammonal (CA)) in rats [29,30]. In mice prenatal DEX exposure reduces hippocampal volume and increases apoptosis, however these effects do not persist until adulthood, whereas it induces a permanent proliferation deficit in the adult hippocampus [28]. In rats, prenatal, but not postnatal DEX increases cleaved caspase-3, a marker of apoptosis, in the CA1 and CA3 regions of the hippocampus, particularly in females [27].

Some studies suggest that the effects of prenatal DEX exposure can be both detrimental and protective within the same organism. For example, one study reports prenatal exposure to DEX prevents the increased vascularization observed in the amygdala after chronic stress exposure but exacerbates the retraction of vascularization in the hippocampus [19]. In another study, fetal exposure to DEX in mice resulted in a decrease in blood vessel density and impaired blood-brain barrier in the hypothalamus, whereas it enhances the barrier integrity in the cortex [31].

Furthermore, prenatal exposure to DEX in rats correlates with an increased volume and increased dendritic length of the bed nucleus of the stria terminalis (BNST), which is involved in fear responses. In contrast, prenatal exposure to DEX results in reduced volume of the amygdala due to dendritic length diminution [17]. In addition, prenatal DEX exposure in rats is also associated with a smaller volume and reduced cell number in the nucleus accumbens (NAcc), a heterogenous structure belonging to the striatum which is involved in the “reward pathway” and drug addiction [32]. Interestingly, this effect is stronger in males than females, which may be relevant to the increased vulnerability of males to drug addiction

[33]. Thus, prenatal DEX exposure affects the development of the brain, and the effect can be negative or positive depending on the specific region of the brain.

Prenatal sGC exposure and HPA axis regulation

Several studies suggest that prenatal DEX exposure alters GR expression in the brain, in association with changes in HPA axis regulation [18]. In marmosets, late gestation DEX exposure leads to a decrease in GR mRNA expression but no effect on MR expression in the PFC, both in neonates and in adulthood [34]. In rats, prenatal DEX decreases GR expression in the hippocampus [35], whereas in guinea pigs GR expression is decreased [36]. In addition, following a 1 hour-recovery from a restraint stress, prenatally DEX-exposed rats demonstrate reduced GR mRNA expression in the pituitary gland, whilst control rats have increased GR mRNA expression [37]. Moreover, in rats and guinea pigs, offspring of DEX-treated dams have increased mRNA levels of the corticotrophin-releasing hormone (CRH), a critical coordinator of the HPA axis, in the PVN of the hypothalamus [35–37], indicating that prenatal DEX leads to persistent changes in HPA axis regulation. CRH is normally released by the hypothalamus following a stressor, and acts synergistically with arginine vasopressin (AVP) to induce the production of adrenocorticotrophic hormone (ACTH) by the pituitary. ACTH then stimulates the production of corticosterone by the adrenal gland, inducing the negative feedback loop (Figure 1).

In rats, maternal adrenalectomy, which removes endogenous glucocorticoids, induces a depression-like phenotype in the offspring when assessed using the OFT and FST and is associated with increased GR expression in the hippocampus and decreased GR expression in the hypothalamus [38]. Whilst supplementation with high-dose corticosterone (CORT) following adrenalectomy during pregnancy restores normal GR expression in the hippocampus, it still results in HPA axis dysregulation. Interestingly, there is a sex-specific effect in that although both sexes had an exaggerated plasma ACTH response to stress, only females show a reversal of the effect following high dose CORT substitution [38].

Prenatal sGC exposure and the neurotransmitters

Several neurotransmitters are important for HPA axis regulation and mood, and are altered following prenatal sGC exposure, including the serotonergic, dopaminergic and GABAergic systems (Figure 2). Prenatal DEX treatment leads to decreased serotonin (5-HT) concentrations in the hippocampus, decreased mRNA expression of the serotonin receptor 5-

HT1A-R in the PFC and decreased protein expression of brain-derived neurotrophic factor (BDNF), which is implicated in the pathogenesis of depression [39], in the PFC and hippocampus. Interestingly, early intervention with fluoxetine reverses the dysregulation of 5-HT signaling as well as the behavioral phenotype in the prenatally DEX-exposed offspring [39]. However, a separate study showed that prenatal DEX induces an increase in 5-HT in the cortex and hippocampus [40]. The discrepancies might be explained by the different timing of DEX exposure between studies, e.g. exposure to DEX daily throughout pregnancy [40] versus exposure only during the final week of gestation [41]. In addition, the depressive-like phenotype induced by prenatal DEX exposure in rats correlates with a decrease in dopamine (DA) in the NAcc and amygdala and this is partially rescued by a treatment with L-3,4-dihydroxyphenylacetic acid (L-DOPA) [42]. Finally, prenatal DEX exposure in rats decreases calretinin expression, a calcium binding protein (CBP) that is expressed in GABAergic neurons, in the amygdala of adult female, but not male offspring [24].

Epigenetic dysregulation and prenatal programming of the brain

The mechanisms by which the effects of prenatal exposure to stressors are established and sustained are not well understood. Epigenetic dysregulation may provide a plausible link between early life adversity (including sGC exposure) and sustained alterations in gene expression that lead to adulthood diseases [43]. Recent studies suggest epigenetic mechanisms such as DNA methylation or histone modifications are involved in the development of neuropsychiatric disorders [44,45]. In addition, the developmental period is a time when major epigenetic remodelling events occur [46], especially in the brain [47], which renders it a time of particular susceptibility to epigenetic re-programming,.

Animal studies suggest that prenatal GC overexposure can induce epigenetic changes in a number of candidate genes implicated in neuropsychiatric disorders, including the glutamic acid decarboxylase (GAD67), reelin and BDNF genes [48–50]. Prenatal DEX exposure in rats leads to a reduction in the protein and mRNA levels of reelin and GAD67 and associates with an overexpression of DNA methyltransferase 1 (DNMT1) in the hippocampus [49]. Melatonin restores the level of reelin and GAD67 by reducing DNMT1 mRNA expression and thus reducing the binding of DNMT1 and methyl-CpG binding protein 2 (MeCP2) to the reelin promoter. This result is supported by another study showing that an increase in DNMT1 following maternal stress leads to hypermethylation at specific CpG-rich regions of the GAD67 and reelin promoters, associated with decreased expression of both genes [50].

Similar results were obtained in another recent study which reported that prenatal stress leads to decreased cortical expression of reelin, accompanied by hypermethylation at the reelin promoter region. These molecular changes were associated with persistent behavioral consequences in adults, such as increased spontaneous locomotor activity, high anxiety levels and cognitive deficits [48]. sGC treatment during late gestation in guinea pigs induces genome-wide epigenetic changes in promoter methylation and histone acetylation in the fetal hippocampus [51,52]. The same group also reported changes in the developmental trajectory of DNA methylation in several other fetal tissues in prenatally exposed guinea pigs, which has been associated with altered expression of genes involved in the DNA methylation process. Furthermore, these effects persisted into adulthood and were transmitted to the next generation [52].

Conclusions and suggestions for further work

Prenatal sGC exposure associates with altered behavior and increased neurodevelopmental disease risk. Importantly, these effects depend highly on the timing of exposure and may be sex-specific [24,32,38] with females being more susceptible to stress and a depression-like phenotype than males [11,53]. Additionally, the discrepancies between studies using the same behavioral tests in animal models suggests that other factors such as animal housing and external stressors can affect the outcome [54,55]. There is increasing evidence suggesting a role for epigenetic dysregulation in prenatal sGC programming; future *in vivo* studies could be designed to elucidate the consequences of prenatal sGC on the level/activity of epigenetic regulators, e.g. the Dnmts, the ten-eleven-translocase (TET) proteins that are involved in the generation of cytosine 5-hydroxymethylation (5hmC) and the polycomb-group proteins regulating histone marks. Further, studies which delineate the effects on genome wide patterns of 5-methylcytosine, 5hmC and histone modifications in dissected brain regions will help to elucidate the mechanisms by which prenatal sGC exposure induces long-lasting effects on the phenotype.

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Figure 1: **The different components of the brain and their roles in behaviour and the HPA axis negative feed-back loop (in italic**s). CRH: Corticotrophin Realease Hormone, ACTH: AdrenoCorticoTropic Hormone, CORT: corticosterone, PFC: Pre-frontal cortex, NAcc: Nucleus Accumbens.

Figure 2: **Neurotransmitter pathways implicated in mood balance and dysregulation in the offspring from DEX-treated mothers (black arrow).**

Table1: Behavioural tests and the consequences of prenatal DEX exposure

Test	Measure	Description	Prenatal DEX effects	References
Open Field Test	Anxiety	Large open area with wall where the locomotion and the willingness of the animal to explore is measured	decreased locomotion and exploration activity	17.18.19
			no differences	Zeng et al
Elevated plus maze	Anxiety	The test setting consists of a plus-shaped apparatus with two open and two enclosed arms, each with an open roof, elevated 40–70 cm from the floor. The model is based on rodents' aversion of open spaces.	Animals spend less time in the open arms	16.18
			no differences	Zeng et al
Morris water maze	spatial learning and memory	The rat is placed in a large circular pool and is supposed to find an invisible or visible platform that allows it to escape the water by using various cues	animal slower the first day of trial then faster to locate the platform	Zeng et al
			no differences	18
Force Swim Test	depression	Animals are forced to swim in an acrylic glass cylinder fill with water several times.	increased immobility	15.18
			no differences	17

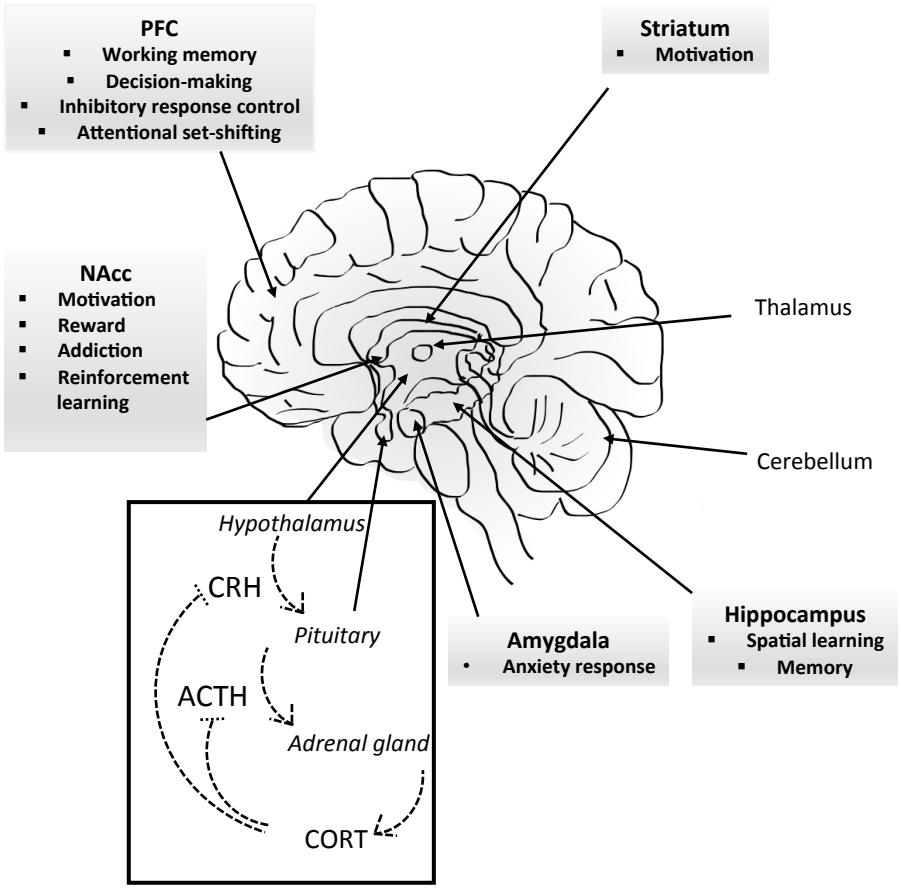
PFC
Working memory
Decision-making
Inhibitory response control
Attentional set-shifting
Striatum
Motivation
NAcc
Motivation
Reward
Addiction
Reinforcement learning
Thalamus
Cerebellum
Hypothalamus
CRH
Pituitary
ACTH
Adrenal gland
CORT
Amygdala
Anxiety response
Hippocampus
Spatial learning
Memory

Figure 1

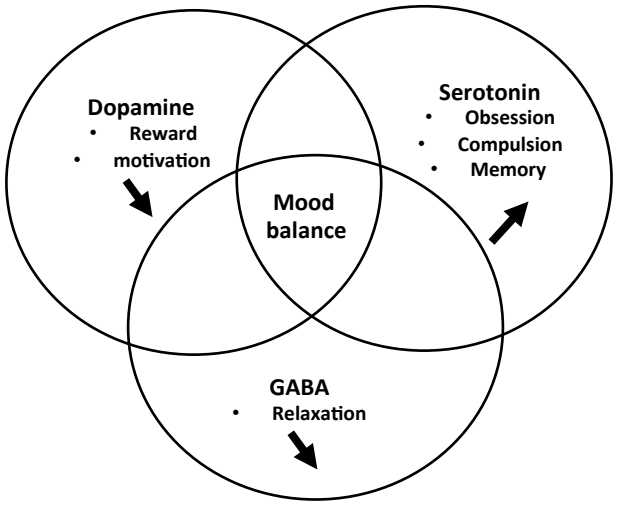
Dopamine
• Reward
• motivation
Serotonin
• Obsession
• Compulsion
• Memory
Mood balance
GABA
• Relaxation

Figure 2